Merging zones approach in a flow-based platform for the determination of the total protein content in microbiological samples

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Lactic acid bacteria (LAB) can produce bacteriocins, that are a portion of the total protein content produced during fermentation. Bacteriocins are peptides or proteins synthesized by bacteria and archaea with a bacteriostatic or bactericidal activity over principally closely related species (e.g., *Listeria monocytogenes*) [1]. These bacteriocins have a wide variety of applications. From the food industry, where bacteriocins can be incorporated in food products susceptible to microbial contamination, reducing the probability of consumer's infection by some of the most prominent pathogenic bacteria; to pharmaceutical industry, where these peptides can be used as a viable alternative to antibiotics due to the emergence of multiple antibiotic-resistant bacteria [2]. Thus, it is important to have a tool to monitor the production of the protein content that is being produced in the fermentation process.

Flow-based methods are well known for their efficiency in the execution of several analytical steps. These systems have started as automation tools for wet chemical analysis and further used in biochemical assays [3]. In this context, an automatic methodology in a flow injection analysis (FIA) platform based on a Lowry assay for the determination of the total protein content produced by lactic acid bacteria (LAB) was developed. This methodology is based in two steps: i) binding of Cu2+ to the protein, resulting in the reduction of the Cu^{2+} to Cu(I); and ii) oxidation of Cu(I) by the Folin-Ciocalteu reagent (FCr). In the last step, the FCr is reduced producing a colour change that can be monitored at 750 nm [4,5].

With the developed methodology it was possible to establish a linear relationship up to 0.5 g/L of BSA (model protein) with a limit of detection of 0.05 g/L. A low consumption of sample and reagents was also achieved by using a merging zones approach with a determination rate of about 90 determinations per hour.

Since the matrix of interest, fermented culture media, is very complex, the possible interferences from the compounds of the chemically defined culture medium will be evaluated and the system will be applied to the quantification of the total protein content produced in a chemically defined media.

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